Immunization with interleukin-2-secreting allogeneic mouse fibroblasts expressing melanoma-associated antigens prolongs the survival of mice with melanoma.

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The survival of C57BL/6 mice (H-2b) bearing B16 melanoma (H-2b) was prolonged if the animals were treated solely by immunization with an interleukin-2 (IL-2)-secreting allogeneic cell construct that expressed melanoma-associated antigens (MAA) along with major histocompatibility complex (MHC) class-I determinants (H-2k; RLBA-IL-2 cells). This was the case if the mice were immunized simultaneously with, or 6 days following, the injection of viable B16 cells. Under similar conditions, the survival of tumor-bearing mice immunized with an allogeneic cell construct (H-2k) that expressed MAA but did not secrete IL-2 (RLBA-ZipNeo cells), or with an allogeneic construct (H-2k) that secreted IL-2 but did not form MAA (LM-IL-2 cells), was also prolonged. However, in these instances, the period of survival was significantly shorter than that of mice immunized with the cell construct that combined IL-2 secretion with the expression of MAA. Tumor-bearing mice immunized with non-transfected LM(TK-) cells (H-2k), or irradiated B16 cells (H-2b) failed to survive longer than untreated mice. Although the survival of the treated animals was prolonged, in most instances tumor growth recurred. The recurrent tumors in mice treated with the allogeneic cell constructs formed melanin and were histologically indistinguishable from tumors in untreated mice. Cells from the recurrent tumors were resistant to further immunotherapy and to cytotoxic effector cells obtained from the spleens of mice immunized with the same cellular immunogen used initially. The injection of IL-2-secreting syngeneic B16 cells into C57BL/6 mice invariably resulted in the appearance of non-IL-2-secreting melanomas. Under similar circumstances, tumors failed to develop in C57BL/6 mice injected with IL-2-secreting, or non-secreting, allogeneic cell constructs. Thus, the expression of allogeneic antigens protected the mice from growth of the cellular immunogens.

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1: J Immunother Emphasis Tumor Immunol 1994 Jul;16 (1):24-35

Immunization of mice with allogeneic fibroblasts genetically modified for interleukin-2-secretion and expression of melanoma-associated antigens stimulate predetermined classes of anti-melanoma effector cells.

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Immunization of C57BL/6 mice (H-2b) with a mouse fibroblast cell-line of C3H origin (H-2k) genetically modified for interleukin-2 (IL-2)-secretion and the expression of melanoma-associated antigens (MAAs) (RLBA-IL-2 cells) resulted in a systemic anti-melanoma cellular immune response that led to a prolongation of survival of mice with established melanoma. Here we report certain of the effector cell-types activated for anti-melanoma immunity in mice immunized with the modified cells and, for comparison, the anti-melanoma cell-types activated following immunization with IL-2-secreting, MAA-negative fibroblasts (LM-IL-2 cells) or with non-IL-2-secreting, MAA-positive fibroblasts (RLBA-ZipNeo cells). The data indicate that both Lyt-2.2+ (CD8+) and natural killer/lymphokine-activated killer (NK/LAK) cells with anti-melanoma cytotoxicity were predominant in mice immunized with RLBA-IL-2 cells. NK/LAK cells alone were predominant in mice immunized with LM-IL-2 cells, and Lyt-2.2+ cells were predominant in mice immunized with RLBA-ZipNeo cells. The involvement of L3T4+ (CD4+) cells in the effector phase of the response was not detected in mice immunized with the genetically modified cells. Immunization of mice with both LM-IL-2 cells and RLBA-ZipNeo cells resulted in an anti-melanoma response of greater magnitude than was present in mice immunized with either cell-construct alone. It was equivalent to the melanoma immunity in mice immunized with RLBA-IL-2 cells. These data indicate that the immunogenic properties of the modified cells determined the anti-melanoma effector cell-types and suggest that combination immunotherapy with cell-constructs that stimulate different classes of effector cells may be more effective in immune-mediated tumor regression than immunization with a construct that activates a single effector cell-type alone.

1: Int J Cancer 1992 May 8;51(2):283-9 Related Articles, Link

Immunity to B16 melanoma in mice immunized with IL-2-secreting allogeneic mouse fibroblasts expressing melanoma-associated antigens.

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Co-presentation of weak tumour-associated antigens along with strongly immunogenic determinants leads to the development of an anti-tumour immune response in recipients syngeneic with the tumour. Tumour immunity develops in mice immunized with tumour cells modified by the introduction of cDNA for interleukin-2 (IL-2). Here, we report the anti-tumour response following immunization with an IL-2-secreting cell construct that expresses tumour-associated antigens, along with allogeneic major histocompatibility antigens. The construct was prepared by transfecting LM(TK-) mouse fibroblasts (H-2k) with genomic DNA from B16 melanoma cells syngeneic in C57BL/6J mice (H-2b). Transfectants expressing melanoma-associated antigens (MAA) were then infected with an expression-competent retroviral vector containing a cDNA specifying human IL-2. Cytotoxicity toward B16 cells was detected for as long as 5 months in both spleen and macrophage cell populations in C57BL/6J mice immunized with the IL-2-secreting cells. Mice immunized with non-IL-2-secreting, MAA-positive allogeneic cells developed melanoma immunity as well, but to a lesser extent. Immunity to 2 tumour-cell lines expressing the H-2d haplotype and to YAC-1 cells was detected in peritoneal macrophages, but not in spleen cells from C57BL/6J mice immunized with the cell construct, indicating that the response to B16 cells was only partially specific. C57BL/6J mice immunized with the IL-2-secreting cell construct survived significantly longer, following an injection of viable B16 cells, than mice in various control groups. The contribution of allogeneic antigens to the melanoma immunity was indicated by the failure of mice syngeneic with LM(TK-) cells to develop melanoma immunity following immunization with non-IL-2-secreting, MAA-positive cell constructs. The formation of IL-2 partially compensated for the lack of allogeneic antigens.

PMID: 1533203 [PubMed - indexed for MEDLINE]

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L2: Entry 32 of 43 File: USPT May 12, 1998

US-PAT-NO: 5750102
DOCUMENT-IDENTIFIER: US 5750102 A

TITLE: Double transfectants of the MHC genes as cellular vaccines for immuno prevention of tumor metastasis

DATE-ISSUED: May 12, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Eisenbach; Lea	Rehovot			IL
Feldman; Michael	Rehovot			IL

US-CL-CURRENT: 424/93.21; 424/93.7, 435/325, 435/347, 435/366, 435/69.6

CLAIMS:

We claim:

1. A cellular composition that provokes an immune response in a human patient when said cellular composition is used to treat a patient having a heterozygous haplotype, said cellular composition comprising:

(a) tumor cells isolated from said patient to be treated into which at least two genes encoding MHC proteins of different haplotypes have been inserted, wherein said genes are expressed in said tumor cells and at least one of said MHC proteins has the same haplotype as a haplotype of the heterozygous patient to be treated; and

(b) a pharmaceutically acceptable carrier.

2. The cellular composition according to claim 1 wherein said genes inserted into said tumor cells have been introduced on a single expression vector.

3. The cellular composition according to claim 2 wherein said expression vector is selected from the group consisting of a plasmid and a retroviral vector.

4. The cellular composition according to claim 1 wherein said genes inserted into said tumor cells have been introduced on different expression vectors.

5. The cellular composition of according to claim 4 wherein at least one of said expression vectors is selected from the group consisting of a plasmid and a retroviral vector.

6. The cellular composition according to claim 1, wherein said genes have been integrated into a chromosome of said tumor cell.

7. The cellular composition according to claim 1, wherein said genes are episomally retained in said tumor cell.

8. The cellular composition according to claim 1, wherein the tumor cells have been inactivated and thereby rendered nonreplicating by a treatment, said treatment comprising at least one treatment selected from the group consisting of irradiation inactivation and mitomycin C inactivation.

9. The cellular composition according to claim 1, wherein said genes have been inserted into said tumor cells by transfection.

10. The cellular composition of claim 1 wherein said composition comprises 1.times.10.sup.6 to 1.times.10.sup.9 tumor cells.

11. The cellular composition of claim 10 wherein said composition comprises 1.times.10.sup.7 tumor cells.

12. The cellular composition of claim 1 wherein said composition is formulated as an injectable solution.

13. The cellular composition of claim 1 wherein said MHC proteins are selected from the group of class I MHC molecules HLA-A, HLA-B, and HLA-C.

14. A method of treating a patient suffering from a tumorous disease comprising

administering the cellular composition according to claim 1 to said patient .

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L1: Entry 1 of 1 File: USPT Feb 13, 2001

US-PAT-NO: 6187307
DOCUMENT-IDENTIFIER: US 6187307 B1

TITLE: Cancer immunotherapy with semi-allogeneic cells

DATE-ISSUED: February 13, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cohen; Edward P.	Chicago	IL		

US-CL-CURRENT: 424/93.21; 424/93.71, 435/325, 435/366, 435/372, 435/455, 536/23.5

CLAIMS:

What is claimed is:

1. A semi-allogeneic immunogenic cell for administration to an animal recipient, which comprises an antigen-presenting cell expressing at least one class I MHC or class II MHC determinant that is syngeneic to the recipient and at least one class I or class II MHC determinant that is allogeneic to the recipient, wherein said antigen presenting cell is transformed with and expresses DNA coding for at least one antigen, and wherein said antigen or a part thereof, when complexed with said MHC class I or class II determinant at the cell surface, is recognized by T cells.

2. A semi-allogeneic immunogenic cell for administration to an animal recipient, which comprises an antigen-presenting cell expressing at least one class I MHC or class II MHC determinant that is syngeneic to the recipient and at least one class I or class II MHC determinant that is allogeneic to the recipient and wherein said antigen presenting cell is transformed with and expresses DNA isolated from a neoplasm or a tumor of the recipient.

3. The semi-allogeneic immunogenic cell of claim 1 or 2, wherein said antigen presenting cell is further transformed with a coding sequence for at least one cytokine.

4. The semi-allogeneic immunogenic cell of claim 3 wherein the cytokine is selected from the group consisting of interleukin-1, interleukin-2, interleukin-3, interleukin-4, interleukin-5, interleukin-6, interleukin-7, interleukin-8, interleukin-9, interleukin-10, interleukin-11, interleukin-12, interferon-.alpha., interferon-.gamma., tumor necrosis factor, granulocyte macrophage colony stimulating factor, and granulocyte colony stimulating factor.

5. The semi-allogeneic immunogenic cell of claim 1 or 2, wherein the antigen-presenting cell is selected from the group consisting of a fibroblast, a macrophage, a B cell, and a dendritic cell.

6. The semi-allogeneic immunogenic cell of claim 2, wherein the neoplasm is selected from the group consisting of melanoma, lymphoma, plasmocytoma, sarcoma, glioma, thymoma, leukemias, breast cancer, prostate cancer, colon cancer, esophageal cancer, brain cancer, lung cancer, ovary cancer, cervical cancer, and hepatoma.

7. The semi-allogeneic immunogenic cell of claim 2 wherein the DNA isolated from a neoplasm or tumor comprises coding sequences for tumor associated antigens.

8. The semi-allogeneic immunogenic cell of claim 2 wherein the DNA isolated from neoplastic cells comprises coding sequences for tumor associated antigens that are associated with a tumor, wherein said tumor is selected from the group consisting of melanoma, lymphoma, plasmocytoma, sarcoma, glioma, thymoma, leukemias, breast cancer, prostate cancer, colon cancer, esophageal cancer, brain cancer, lung cancer, ovary cancer, cervical cancer, and hepatoma.

9. A therapeutic composition comprising the semi-allogeneic immunogenic cell of at least one of claims 1, 2, 7, or 8 admixed with a therapeutically acceptable carrier.

10. A therapeutic composition comprising the semi-allogeneic immunogenic cell of claim 3 admixed with a therapeutically acceptable carrier.

11. A therapeutic composition comprising the semi-allogeneic immunogenic cell of claim 4 admixed with a therapeutically acceptable carrier.

12. A therapeutic composition comprising the semi-allogeneic immunogenic cell of claim 5 admixed with a therapeutically acceptable carrier.

13. A therapeutic composition comprising the semi-allogeneic immunogenic cell of claim 6 admixed with a therapeutically acceptable carrier.

14. A semi-allogeneic immunogenic cell for administration to an animal recipient, which comprises an antigen-presenting cell expressing at least one of class I or class II MHC determinants, wherein said antigen presenting cell is genetically selected such that at least one of said class I MHC or class II MHC determinants is syngeneic to the recipient and at least one of said class I or class II MHC determinants is allogeneic to the recipient, wherein said antigen presenting cell expresses at least one antigen, and wherein said antigen or a part thereof, when complexed with said MHC class I or class II determinant at the cell surface, is recognized by T cells.

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L2: Entry 25 of 43 File: USPT May 16, 2000

US-PAT-NO: 6063375
DOCUMENT-IDENTIFIER: US 6063375 A

TITLE: Semiallogeneic cell hybrids and related methods for treating cancer

DATE-ISSUED: May 16, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Gattoni-Celli; Sebastiano	Mt. Pleasant	SC		
Newton, III; Danforth A.	Beaufort	SC		
McClay; Edward F.	Folly Beach	SC		

US-CL-CURRENT: 424/93.21; 435/325, 435/346

CLAIMS:

What is claimed is:

1. A cell designated FO-1 #12, deposited with the American Type Culture Collection under accession Number ATTCC CRL-12177.

2. A cell hybrid formed by the fusion of the cell of claim 1 and a mammalian cell.

3. The cell hybrid of claim 2, wherein the hybrid is irradiated.

4. The cell hybrid of claim 2, wherein the mammalian cell is a patient-derived human cell.

5. The cell hybrid of claim 2, wherein the mammalian cell is a patient-derived tumor cell.

6. The cell hybrid of claim 5, in the patient-derived tumor cell is a melanoma cell.

7. The cell hybrid of claim 5, wherein the patient-derived tumor cell is a prostatic carcinoma cell.

8. The cell hybrid of claim 5, wherein the patient-derived tumor cell is a colon carcinoma cell.

9. The cell hybrid of claim 5, wherein the patient-derived tumor cell is a lung carcinoma cell.

10. The cell hybrid of claim 5, wherein the patient-derived tumor cell is a breast carcinoma cell.

11. The cell hybrid of claim 5, wherein the patient-derived tumor cell is a pancreatic carcinoma cell.

12. The cell hybrid of claim 4, wherein the patient-derived cell is a white blood cell.

13. A method of treating a solid tumor in a patient, comprising administering to the patient by intradermal injection the irradiated cell hybrid of claim 5, wherein the patient-derived tumor cell is derived from the patient being treated and wherein said administration results in a decrease in growth of said solid tumor.

14. The method of claim 13, wherein the cell hybrid is administered in conjunction with a cytokine.

15. The method of claim 13, wherein the cytokine is IL-2.

16. The method of claim 13, wherein the cytokine is granulocyte-macrophage colony-stimulating factor.

17. The method of claim wherein the cytokine is IL-12.

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L1: Entry 1 of 7 File: USPT Feb 27, 2001

US-PAT-NO: 6194205
DOCUMENT-IDENTIFIER: US 6194205 B1

TITLE: Method for the stimulation of T cells having a desired antigen specificity

DATE-ISSUED: February 27, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Staege; Martin	Munich			DE
Kempkes; Bettina	Munich			DE
Bornkamm; Georg W.	Munich			DE
Hammerschmidt; Wolfgang	Munich			DE
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Polack; Axel	Munich			DE

US-CL-CURRENT: 435/373; 435/372.3, 435/375, 435/467

CLAIMS:

What is claimed is:

1. A method for the stimulation of T cells having a desired antigen specificity, said method comprising:

(a) introducing immortalizing genes into antigen-presenting cells in a manner that permits the regulation of the expression of at least one of these genes and/or the function of the product of that gene to achieve conditionally immortalized antigen-presenting cells;

(b) introducing a gene encoding the desired antigen into the conditionally immortalized cells obtained in step (a) in a manner that permits the expression of the antigen after stopping the expression and/or abolishing the function of at least one of the immortalizing genes or gene products;

(c) expanding the conditionally immortalized antigen-presenting cells by expression of the immortalizing genes and/or by functional activation of the immortalizing gene product;

(d) completing the proliferation of the immortalized antigen-presenting cells by stopping the expression and/or abolishing the function of at least one of the controllable immortalizing genes or gene products;

(e) continuing the expression of the antigen; and

(f) adding leukocytic cells including at least T cells and cultivating the cell mixture to stimulate the T cells directed against the desired antigen.

2. A method in accordance with claim 1, further comprising purifying and isolating the T cells stimulated by step (f).

3. A method in accordance with claim 1, in which the added antigen-presenting cells are obtained from a first donor and in step (f) the allogenic leukocytic cells employed are obtained from a second donor who is syngenic for at least one corresponding MHC molecule serving for antigen presentation with the first donor.

4. A method in accordance with claim 1, in which the antigen-presenting cells of step (a) and the leukocytic cells of step (f) are obtained from a common donor.

5. A method for the stimulation of T cells having a desired antigen specificity, said method comprising:

(a) introducing immortalizing genes into antigen-presenting cells in a manner that permits the regulation of the expression of at least one of these genes and/or the function of that gene product to achieve conditionally immortalized antigen-presenting cells;

(b) introducing a gene encoding the desired antigen into the conditionally immortalized cells obtained in step (a) in a manner that permits expression of the antigen after stopping the expression and/or abolishing the function of at least one of the immortalizing genes or gene products;

(c) expanding the conditionally immortalized antigen-presenting cells by expression of the immortalizing genes and/or by functional activation of the immortalizing gene product;

(d) adding leukocytic cells including at least T cells to a first portion of the immortalized cells obtained in steps (a) through (c) expressing the desired antigen and culturing the cell mixture to stimulate the T cells directed against the desired antigen;

(e) completing the proliferation of a second portion of the immortalized cells obtained in steps (a) through (c) by stopping the expression and/or abolishing the function of at least one of the controllable immortalizing genes or gene products required for immortalization; and

(f) co-cultivating the antigen-presenting cells after stopping the expression and/or abolishing the function of at least one of the controllable immortalizing genes or gene products of (e) with the T cells stimulated in step (d) for restimulation of the T cells directed against the desired antigen.

6. A method in accordance with claim 5, further comprising purifying and isolating the T cells stimulated by step (f).

7. A method in accordance with claim 5, in which the added antigen-presenting cells are obtained from a first donor and in step (d) the allogenic leukocytic cells employed are obtained from a second donor who is syngenic for at least one corresponding MHC molecule serving for antigen presentation with the first donor.

8. A method in accordance with claim 5, in which the antigen-presenting cells of step (a) and the leukocytic cells of step (d) are obtained from a common donor.

9. A method in accordance with claims 1 or 5, in which said immortalizing genes are genes selected from the group consisting of Epstein-Barr virus gènes, adenoviral genes, and oncogenes.

10. A method in accordance with claims 1 or 5, in which the vectors for the introduction of the gene encoding the desired antigen into the immortalized antigen-presenting cells are vectors obtained from a virus selected from the group consisting of Epstein-Barr virus, adenoviruses, retroviruses, foamyviruses, poxviruses, and SV40 virus.

11. A method in accordance with claims 1 or 5, in which the immortalizing genes are a member selected from the group consisting of the EBNA2 gene, the EBNA3a gene, the EBNA3b gene, the EBNA3c gene, and the LMP gene of Epstein-Barr virus.

12. A method in accordance with claims 1 or 5, in which the immortalizing genes are genes that may be regulated by hormones or antibiotics.

13. A method in accordance with claims 1 or 5, in which steps (a) and (b) are performed simultaneously.

14. A method in accordance with claims 1 or 5, in which the gene coding the desired antigen is arranged on the same vector that bears the immortalizing genes and in which at least one of the immortalizing genes is controllable.

15. A method in accordance with claims 1 or 5, further comprising, prior to the step of adding leukocytic cells, modulating the immunostimulatory properties of the immortalized cells expressing the desired antigen by culturing said immortalized cells in the presence of a member selected from the group consisting of a CD40 stimulus and cytokines.

16. A method in accordance with claims 1 or 5, in which the antigen-presenting cells are a member selected from the group consisting of B cells, macrophages, dendritic cells, and fibroblasts.

17. A method in accordance with claims 1 or 5, further comprising, prior to the step of adding leukocytic cells, permanently suppressing the growth of the immortalized antigen-presenting cells.

18. A method in accordance with claims 1 or 5, in which said T cells are obtained from mammals.

19. A method in accordance with claims 1 or 5, in which said T cells are obtained from a member selected from the group consisting of humans and rodents.

20. A method for the stimulation of T cells having a desired antigen specificity, said method comprising:

(a) introducing immortalizing genes into antigen-presenting cells in a manner that permits regulation of the expression of at least one of these genes and/or the function of that gene product to achieve conditionally immortalized antigen-presenting cells;

(b) expanding the conditionally immortalized antigen-presenting cells by expression of the immortalizing genes and/or by functional activation of the immortalizing gene product;

(c) completing the proliferation of the immortalized antigen-presenting cells by stopping the expression and/or abolishing the function of at least one of the controllable immortalizing genes or gene products;

(d) introducing a gene encoding the desired antigen into the immortalized cells obtained in step (c) in a manner that permits the expression of the antigen after stopping the expression and/or abolishing the function of at least one of the immortalizing genes or gene products,

(e) immediately after step (d), continuing the expression of the antigen;

(f) adding leukocytic cells including at least T cells and cultivating the cell mixture to stimulate the T cells directed against the desired antigen; and

(g) optionally purifying and isolating the stimulated T cells.

21. A method for the stimulation of T cells having a desired antigen specificity, said method comprising:

(a) introducing immortalizing genes into antigen-presenting cells in a manner that permits regulation of the expression of at least one of these genes and/or the function of that gene product to achieve conditionally immortalized antigen-presenting cells;

(b) expanding the conditionally immortalized antigen-presenting cells by expression of the immortalizing genes and/or by functional activation of the immortalizing gene product;

(c) introducing a gene encoding the desired antigen into the immortalized cells obtained in step (b) in a manner that permits expression of the antigen after stopping the expression and/or abolishing the function of at least one of the immortalizing genes or gene products,

(d) immediately after step (c), adding leukocytic cells including at least T cells to a first portion of the immortalized cells obtained in steps (a) through (c) expressing the desired antigen and culturing the cell mixture to stimulate the T cells directed against the desired antigen;

(e) completing the proliferation of a second portion of the immortalized cells obtained in steps (a) through (c) by stopping the expression and/or abolishing the function of at least one of the controllable immortalizing genes or gene products required for immortalization;

(f) co-cultivating the antigen-presenting cells after stopping the expression and/or abolishing the function of at least one of the controllable immortalizing genes or gene products of (e) with the T cells stimulated in step (d) for restimulation of the T cells directed against the desired antigen; and

(g) optionally purifying and isolating the stimulated T cells.

22. A method for the stimulation of T cells having a desired antigen specificity, said method comprising:

(a) introducing immortalizing genes into antigen-presenting cells in a manner that permits regulation of the expression of at least one of these genes and/or the function of that gene product to achieve conditionally immortalized antigen-presenting cells;

(b) expanding the conditionally immortalized antigen-presenting cells by expression of the immortalizing genes and/or by functional activation of the immortalizing gene product;

(c) introducing a gene encoding the desired antigen into a first portion of the immortalized cells obtained in step (b) in a manner that permits expression of the antigen after stopping the expression and/or abolishing the function of at least one of the immortalizing genes or gene products;

(d) immediately after step (c), adding leukocytic cells including at least T cells to said portion of the immortalized cells obtained in steps (a) through (c) expressing the desired antigen and culturing the cell mixture to stimulate the T cells directed against the desired antigen,

(e) introducing a gene encoding the desired antigen into a second portion of the immortalized cells obtained in step (b) in a manner that permits expression of the antigen after stopping the expression and/or abolishing the function of at least one of the immortalizing genes or gene products;

(f) completing the proliferation of said second portion of the immortalized cells obtained in step (e) by stopping the expression and/or abolishing the function of at least one of the controllable immortalizing genes or gene products required for immortalization;

(g) immediately after step (f), co-cultivating the antigen-presenting cells after stopping the expression and/or abolishing the function of at least one of the controllable immortalizing genes or gene products of (f) with the T cells stimulated in step (d) for re-stimulation of the T cells directed against the desired antigen; and

(h) optionally purifying and isolating the stimulated T cells.